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(54) Process for transesterifying fats.

(57) Fats are advantageously transesterified with an enzymatic preparation containing a lipase having the thermostability at a sufficiently high temperature to melt a reactive substrate, without use of a solvent, water being removed out of the reaction system during the reaction.

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EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	EP-A-0 035 883 (FUJI OIL COMP. LTD) * Claims 1-18; page 20, lines 3-7 *	1,2,4,6	C 11 C 3/10 C 11 C 3/08
Y	EP-A-0 140 542 (NOVO INDUSTRI A/S) * Claims 1-4,15; page 8, lines 12-15; examples 12-14 * & JP-A-60 098 984 (Cat. D)	1,4-6	
Y	EP-A-0 126 416 (ASAHI DENKA KOGYO K.K.) * Claims 1,3,4,9; page 18, lines 2-23 *	1,4-6	
A	* Claims 11,12; page 18, line 24 - page 19, line 18; example 2 * & JP-A-60 019 495 (Cat. D); & JP-A-60 203 196 (Cat. D)	3	
A	GB-A-2 035 359 (FUJI OIL CO. LTD) * Claims 1,9,10 * & JP-A-55 071 797 (Cat. D)	1	
A	EP-A-0 064 855 (UNILEVER) * Claims 1-8,12,16 *	1	TECHNICAL FIELDS SEARCHED (Int. Cl.4) C 11 C C 12 P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	08-06-1989	DEKEIREL M.J.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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(54) Process for transesterifying fats.

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PROCESS FOR TRANSESTERIFYING FATS

This invention relates to a process for transesterifying fats with the use of a lipase. More particularly, it relates to a process for transesterifying fats with the use of an enzymatic preparation containing a lipase which has a thermostability at a sufficiently high temperature to melt the fats to be used as the substrate.

Similar to hydrogenation, transesterification of fats is an important technique in the production of edible processed fats such as margarine or shortening.

Prior Art:

The transesterification of fats has been carried out through chemical processes. Namely, there have been employed alkaline materials such as alkali metal, alkali metal alcholates or alkali metal hydroxides or various metal salts as catalysts therefor. However these conventional methods would result in rearrangement of fatty acids in the fats according to the principle of random distribution with regard to the position where a fatty acid binds hydroxy group of glycerol. No specificity is observed at all in the binding position of the fatty acid in the transesterified fats.

Thus, these conventional chemical methods are nonselective in the binding position of the fatty acids in a glyceride. This would sometimes bring about some improvements in the physical properties of fats in the production of conventional edible processed fats such as margarine or shortening. However these nonselective methods are unsatisfactory for the production of fats having a specific glyceride composition. The term "specific composition" as used herein means, for example, such a composition wherein most of glycerides have a symmetric configuration, as observed in natural cacao butter.

Recently the conventional nonselective methods have been replaced with some newly developed processes for selective transesterification of fats, in order to produce products of specific compositions. Fats are transesterified with a lipase selectively in relation to position. The lipase used is an enxyme being capable of hydrolyzation of fats. See Japanese patent publication A (unexamined) No. 104506/1977. According to this process, it is required that moisture be present in the reaction system in order to activate the lipase. Although the required amount of the moisture is as small as 0.2 to 1.0 %, it is unavoidable that the inherent properties of the lipase induce the hydrolysis of the fats to thereby form by-product, e.g., diglyceride, which lowers the yield of the transesterified product.

Further there has been attempted to lower the moisture content to 0.1 % or below in order to suppress the formation of by-product (cf. Japanese Patent Laid-Open No. 71797/1980). However this process is not advantageous from the practical viewpoint, since the decrease in the moisture content would be substantially accompanied by a decrease in the reaction rate.

Furthermore there has been proposed a process wherein the transesterification is carried out in two steps of degradation and synthesis to increase the reaction rate (cf. Japanese Patent Laid-Open No. 19495/1985 and No. 203196/1985). However it is difficult to control this two-step reaction, in particular, the degradation step. Although it is interesting to note that diglyceride is a major concern in this process, it is technically difficult to selectively obtain diglyceride alone in the decomposition step. Thus it is unavoidable that the diglyceride would further degradate into monoglyceride and/or glycerol and that there still remain a large amount of the original triglyceride. It is further unavoidable that the presence of 1,3-diglyceride formed by nonenzymatic transformation of the diglyceride would lower the yield of an aimed transesterified product in the second, i.e., synthesis step. This problem would become serious with a rise in temperature. In addition, the reaction rate in the second step is unsatisfactory, compared with those of conventional transesterification reactions. Thus it may be concluded that this two-step process unavoidably required complicated operations.

Since conventional enzymatic preparations have an unsatisfactory thermostability, it is required to use a solvent in order to dissolve a substrate, in particular, when said substrate has a high melting point. In order to solve this problem, there has been recently developed a thermostable enzymatic preparation (cf. Japanese Patent Laid-Open No. 98984/1985). However there is another problem that an enzymatic reaction at a relatively high temperature, i.e., 50°C or above without using any solvent would be frequently accompanied by the liberation of the moisture from the enzymatic preparation to the reaction system, which accelerates the formation of by-product, i.e., diglyceride to thereby lower the yield of the aimed product.

As described above, the tranesterification of fats with a lipase has various characteristic and advantageous properties compared with the conventional chemical methods. However there still remain many problems which should be overcome prior to the industrial application of the same.

From the economic point of view, it is desirable to transesterify fats with a thermnostable lipase preparation without using any solvent, since the use of a solvent would lower the productivity and cause an energy loss.

On the other hand, it is necessary to use a small amount of the enzyme or to recover and repeatedly use the same, since enzymes required for a reaction as described above are significantly expensive at present.

Attempts to lower the moisture content in an enzymatic preparation would substantially lower the reaction rate, require an extremely large reaction apparatus and cause a decrease in the production efficiency. Further it is unavoidable that the enzyme would be denatured with the lapse of time, when it is to be recovered and reused. Thus a given amount of the enzyme can give only a limited amount of transesterification products. Thus there has been known to process which gives a satisfactory reaction rate and suppressed formation of diglyceride from the industrial viewpoint, as well as the prolonged use of the enzyme from the economic viewpoint.

Summary of the Invention

Under these circumstances, we have attempted to economically suppress the hydrolysis of fats and to efficiently transesterify the same. As a result of out studies on the transesterification of fats with the use of an enzymatic preparation containing a lipase, which will be simply called a lipase preparation hereinafter, and on the characteristics of the lipase, we have found a process for effectively utilizing the lipase preparation, thus completing the present invention.

Accordingly, the present invention relates to a process for transesterifying fats with the use of a lipase preparation, characterized in that said lipase preparation has a thermostability at a sufficiently high temperature to melt a substrate; that no solvent is used; and that moisture is removed from the reaction system during the reaction.

In other words, the invention provides a process for transesterifying fats with an enzymatic preparation containing a lipase having the thermostability at a sufficiently high temperature to melt a reactive substrate, without use of a solvent, water being removed out of the reaction system during the reaction.

Brief Description of the Drawing:

Fig. 1 is a schematic view of the reactor which is an external circulation reactor provided with a packed column as used in Example 5 wherein:

1 represents a packed column;
2 and 2′ represent each a jacket;
3 represents a receiver;
4 represents a fixed blade;
5 represents a stirring blade;
6 represents a liquid feed pump;
7 represents a flowmeter; and
8 represents a pressure gauge.

The term "transesterification of fats" as used herein includes transesterification between fats and fatty acid or its ester; mutual transesterification between different fats; transesterification between fatty acid ester and fatty acid; and mutual transesterification between different fatty acid esters.

It has been already revealed that a lipase would catalyze not only hydrolysis but also the reverse thereof, i.e., synthesis (cf. M. Iwai, Y. Tsujisaka and J. Fukumoto, J. Gen. Appl. Microbiol., 10, 13 (1964)).

Having studied the transesterification of fats from the enzymochemical and kinetic point of view, taking into account the above shown finding, we have found that a complex of diglyceride and an enzyme participates in the reaction and that the transesterification rate can be expressed by the following equation:

$$V = k(E \bullet DG)(FA)$$

wherein k represents an overall reaction rate constant;
(FA) represents the concentration of fatty acid;
and

(E$_o$DG) represents the concentration of the diglyceride/enzyme complex.

The value of k significantly depends on the moisture content in the reaction system and the concentration of the enzyme. However an increase in only the moisture accelerates only the liberation of diglyceride and can not substantially increase the transesterification rate.

On the other hand, an increase in the concentration of the enzyme would suppress the formation of the diglyceride and, as is obvious from the above equation, increase the transesterification rate. However an increase in only the concentration of the lipase preparation could hardly suppress the formation of the diglyceride, since the moisture contained in said preparation would be readily liberated into the reaction system.

We have paid our attention to the fact that the synthesis capability of a lipase varies depending on the moisture content therein and thus fats can be efficiently transesterified without forming any by-product under a low moisture content condition, thus completing the present invention.

Now the present invention will be described in detail.

The transesterification of fats with the use of a lipase preparation capable of hydrolyzing the fat can be carried out in a single step without requiring any complicated reaction process by employing preferably 5 to 100 part (by weight; the same will apply hereinafter) of said lipase preparation per 100 parts of a substrate mixture and continuously removing moisture from the reaction system from the initiation or in the course of the reaction. Thus the transesterification can be carried out in a single step in a shorter period of time, accompanied by neither any complicated reaction process nor a decrease in the yield of transesterification product caused by the hydrolysis of the substrates. Further the decrease in the transesterifying activity of the lipase preparation can be suppressed thereby, which makes it possible to repeatedly use the preparation.

In the process of the present invention, the type of a reactor is not strictly limited. A conventional batch type reactor provided with a stirrer or a circulation reactor provided with a packed column may be effectively employed from the viewpoint of the dehydration efficiency. Alternately a falling or fluidized bed type continous reactor may be used.

As the lipase preparation to be used in the present invention, thermostable immobilized lipase preparations supported on various carriers are preferable. The lipase preparation is preferably used in an amount of 5 to 100 parts per 100 parts of the fats. The presence of the lipase preparation in an amount exceeding the above upper limit is undesirable since it might cause an increase in the slurry concentration in the reaction system to thereby lower the workability.

Examples of the lipase of the lipase preparation to be used in the present invention include animal lipases such as those originating from microorganisms belonging to the genera Rhizopus, Aspergillus, Chromobacterium, Mucor and Pseudomonas, each having a high positive selectivity; those originating from microorganisms belonging to the genus Candida, each showing no specificity; and pancreatic lipases. Among these lipases, those produced by thermostable strains belonging to the genera Rhizopus, Pseudomonas, Chromobacterium, Mucor and Candida are particularly preferable. The porcine pancreas lipase may be used.

The lipase is preferably immobilized on a known carrier. Any carriers for immobilization, for example, inorganic materials insoluble in the transesterification system, such as Celite, kieselguhr, kaolinite, silica gel, perlite, glass fiber, molecular sieves, activated carbon and calcium carbonate and organic polymers exerting no adverse effect on the lipase activity, such as cellulose powder, ion exchange resins and chitosan may be employed. The carrier may be in various forms such as powder, granule, fiber or sponge. It is further preferable that the thermostability and activity of the enzyme are enhanced by immobilization. Thus it is particularly preferable to use a lipase immobilized on a macroscopically porous anion exchange resin.

The fats to be used in the present invention are selected from among common vegetable and animal oils and fats, processed products thereof and mixtures thereof. Examples thereof are soybean oil, cotton seed oil, rapeseed oil, corn oil, safflower oil, sunflower oil, coconut oil, beef tallow, lard and fish oil. When a cacao butter substitute is to be produced through transesterification, oils and fats mainly comprising glycerides having an oleic acid group bound to the 2-position thereof, such as palm oil, olive oil, sunflower oil containing a large amount of an oleic acid group, safflower oil containing a large amount of an oleic acid group, tsubaki oil, sasanqua oil, sal fat, shea butter, illipe butter, kokum butter, mowrah fat, phulwara butter, Bornean tallow, mango kernel oil and fractionation products thereof may be employed.

The transesterification may be carried out by reacting ester with fatty acid, ester with ester or ester with alcohol. Examples of the fatty acids are straight-chain ones having 2 to 24 carbon atoms and occurring in nature, e.g., saturated fatty acids such as palmitic, stearic and behenic acids as well as unsaturated ones such as oleic, linolic and eicosapentaenoic acids.

In the process of the present invention, the transesterification may be carried out at a temperature of 30 to 90°C, which is somewhat higher than that of general enzymatic reactions. In order to remove moisture from the reaction system, the reaction may be carried out under a reduced pressure lower than the vapor pressure. It is perferable that the reduced pressure may be within a range of 3 to 150 mmHg, although it is not restricted thereto.

It is preferable to adjust the moisture content in the reaction system at the completion of the reaction to 0.03 part per part of the fat used as the substrate, from the viewpoint of suppressing the formation of diglyceride. Since rapid dehydration may lower the reaction rate, dehydration may be carried out at a rate of 1.0×10^{-3} to 1.0×10^{-2} g/g of substrate/hr from the viewpoint of maintaining a desirable reaction rate, although it is not restricted thereto. It is also possible to blow an inert gas such as dry nitrogen into the reaction system to thereby remove moisture therefrom, if required.

From the reaction mixture thus transesterified, fatty acid, a small amount of partial ester such as monoglyceride and diglyceride and unreacted alcohol may be readily removed by any conventional purification procedure such as liquid/liquid extraction, neutralization with an alkali or vacuum or molecular-distillation. Thus the transesterification product can be obtained in a pure form.

Effects of the Invention:

The process of the present invention aims at allowing a lipase to fully exhibit its synthesis activity by taking advantage of the fact that the synthesis activity of lipase is directly proportional to the concentration of the enzyme and inversely proportional to the moisture content thereof.

It is the largest effect of the present invention that the formation of by-product can be suppressed without lowering the reaction rate by removing moisture, which would accelerate the formation of the by-product, from the reaction system from the initiation or in the course of the reaction, when a sufficiently large amount of a lipase is used based on the amount of the substrate.

The present invention exhibits an additional effect such that a sufficiently high concentration of a lipase preparation would result in a mutually stabilizing effect of enzymes, which lowers the denaturation of the enzymes with the lapse of time. Thus the lipase preparation recovered after the reaction can be effectively reused, which significantly enhances the productivity per unit weight of the lipase when this process is carried out on an industrial scale. Thus the process of the present invention brings about an improvement in economy. In addition, the process of the present invention may be applied to, for example, the production of a substitute for expensive cacao butter from inexpensive palm oil with the use of a positionally selective lipase preparation.

Example:

To further illustrate the present invention, and not by way of limitation, the following Examples will be given.

Example 1

To 100 parts of a medium-melting fraction of palm oil having an iodine value of 30.5 and comprising 4.6 % of diglycerides and 100 parts of commercially available stearic acid of a purity of 93 % (Lunac S-90; mfd. by Kao Corporation), 30 parts of a commercially available thermostable immobilized enzyme (mfd. by Novo Industri A.S.), which comprised a lipase originating from Mucor miehei immobilized on a macroscopically porous anion exchange resin and contained 8.0 % of moisture, was added and the resulting mixture was allowed to react at 60°C under a pressure of 150 mmHg. At the starting point, the reaction system contained 0.045 part of moisture per part of the reaction materials. After the completion of the reaction, the moisture content in the reaction system was 0.004 part.

After the completion of the reaction, the products were recovered and a triglyceride fraction was collected by chromatography with the use of a silica gel column (mfd. by Merck, #7735) (developing solvent: n-hexane/ethyl ether (90 : 10)). The triglyceride fraction thus collected was converted into methyl esters according to the Standard Method for Analyzing Fats and Oils and the alkyl group composition thereof was analyzed by gas chromatography. The reaction ratio was calculated from the amount of the stearic acid incorporated into the triglycerides according to the following equation by taking the equilibrium

value as 100 %, to thereby examine the progress of the transesterification:
reaction ratio (%) after t hr = 100 × (St - So)/(S∞ -So)

wherein St represents the stearic acid content in the fats t hours after the initiation of the reaction; So represents the stearic acid content in the starting materials; and

S∞ represents the stearic acid content at 1,3-random equilibrium.

The reaction ratio thus calculated was 91.8 %, suggesting that the reaction proceeded sufficiently.

After the completion of the reaction, fatty acids were removed from the reaction mixture by column chromatography with the use of a Florisil column (mfd. by Merck; #12518) and the glyceride composition of the residue was analyzed with reverse phase high perfomance liquid chromatography (ODS Silica : Hitachi Gel # 3750, eluent:
acetone/acetonitrile). As a result, it was found that the diglyceride content after the reaction was 4.8 % suggesting that the substantial increase therein accompanying the reaction was only 0.2 %.

Comparative Example 1

The procedure of Example 1 was followed except that the reaction was carried out under atomospheric pressure. The reaction ratio after five hours was as high as 101 %. However the diglyceride content was extremely high, i.e., 25.2 %, while the yield of the aimed transesterified fat was low and the quality of the same was poor.

Example 2

The procedure of Example 1 was followed except that the reaction was not carried out under reduced pressure but 0.01 v/v/m of dry nitrogen was blown into the head space. According to the same analyses as those described in Example 1, the reaction ratio was 91.4 % while the diglyceride content was 9.6 %, suggesting that satisfactory results were obtained similar to the case of the reaction under reduced pressure.

Example 3

The procedure of Example 2 was followed except that the reaction was carried out under a reduced pressure of 100 mmHg from three hours after the initiation of the reaction.

After five hours, the reaction ratio was 96.4 % while the diglyceride content was 5.6 %, suggesting that satisfactory results were obtained.

Example 4

The procedure of Example 1 was followed except that 50 parts of a commercially available thermostable immobilized enzyme (mfd. by Novo Industri A.S.), which comprised a lipase originating from Mucor miehei immobilized on a macroscopically porous anion exchange resin and had been previously dried in vacuo to reduce the moisture content from 8.0 % to 5.5 %, and that the reaction was carried out at 70°C.

After five hours, the reaction ratio was 98.1 % while the diglyceride content was 4.1 %, i.e., lower than that in the starting materials, suggesting that the synthesis had been effected.

Example 5

The following reaction was carried out in a reactor as shown in Fig. 1.

A commercially available thermostable immobilized enzyme (mfd. by Novo Industri A.S.), which comprised a lipase originating from Mucor miehei immobilized on a macroscopically porous anion exchange resin and contained 8.0 % of moisture, was dried in vacuo to give a moisture content of 6.2 %. 30 g of the immobilized enzyme was packed in a packed column 1 and a mixture of 100 g of a medium-melting fraction of apim oil and 100 g of stearic acid was circulated through said packed column at 65°C.

The flow rate of the mixture was 0.18 cm/sec in terms of the superspace velocity. To the bottom of the packed column 1, a receiver 3 was directly connected for recovering the cirulated solution. The pressure in the receiver 3 was reduced to 160 mmHg. After five hours, the reaction ratio was 90.2 % while the diglyceride content was 9.0 %. The circuration was further continued until eight hours after the initiation of the reaction. Thus the reaction ratio was elevated to 96.9% while the diglyceride content was lowered to 8.1 %.

In the reactor as shown in Fig. 1, 2 and 2' represent each a jacket, 4 represents a fixed blade, 5 represents a stirring blade, 6 represents a liquid feed pump, 7 represents a flowmeter and 8 represents a pressure gauge.

Table 1 shows the results.

Table 1

Reaction time (hr)	Reaction ratio (%)	Diglyceride content (%)
1	54.0	11.2
2	70.7	10.7
3	81.7	10.0
5	90.2	9.0
8	96.9	8.1

Comparative Example 2

The procedure of Example 5 was followed except that the pressure in the receiver was atmospheric. After five hours, the reaction ratio was 90.6% while the diglyceride content, which had been increasing with the lapse of time, reached a significantly high level, i.e., 18.0 %. Table 2 shows the results.

Table 2

Reaction time (hr)	Reaction ratio (%)	Diglyceride content (%)
1	46.6	13.2
2	67.1	14.4
3	79.1	16.3
5	90.6	18.0

These Examples suggest that the rate of transesterification can be maintained simultaneously with suppressing the formation of by-product, i.e., diglyceride by employing an enzymatic preparation containing a thermostable lipase and removing moisture from a reaction system wherein no solvent is used. This process can be carried out on an industrial scale.

Claims

1. A process for transesterifying fats with an enzymatic preparation containing a lipase having the thermostability at a sufficiently high temperature to melt a reactive substrate, without use of a solvent, water being removed out of the reaction system during the reaction.

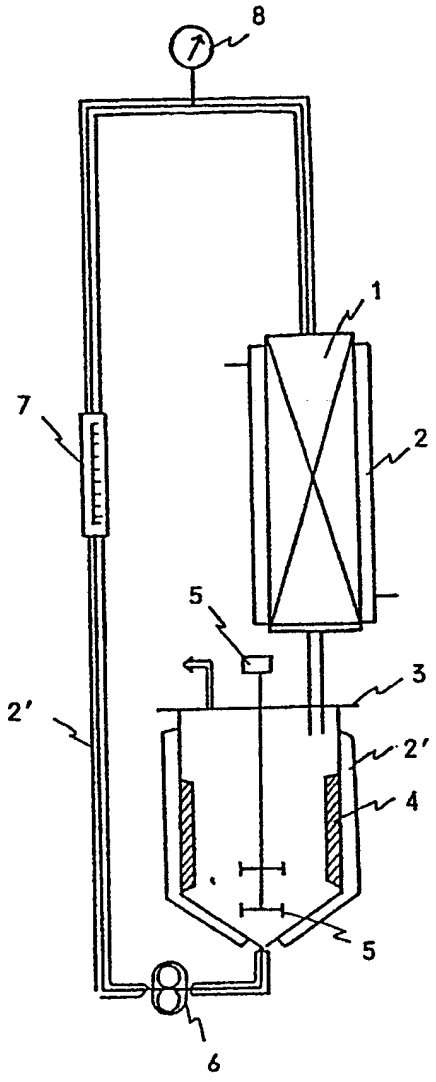
2. A process as claimed in Claim 1, in which the reaction is effected at a reduced pressure to remove away water.

3. A process as claimed in Claim 1, in which the reaction is effected while an inert gas is being introduced into the reaction system to remove away water.

4. A process as claimed in Claim 1, in which the enzymatic preparation is an immobilized enzyme.

5. A process as claimed in Claim 1, in which the enzymatic preparation is a lipase immobilized on a macroscopically porous anion exchanger resin.

6. A process as claimed in Claim 1, in which the lipase has been produced from a thermostable strain belonging to the genus Rhizopus , Pseudomonas, Chromobacterium, Mucor or Candida.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